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The purpose of this project was to examine the influence of parenteral administration of interleukin-1, a cytokine with diverse biological activities, on antibacterial resistance in a laboratory rodent model. We first documented that intraperitoneal injection of minute quantities (0.1-1.0 ng per mouse) of interleukin-1 resulted in a rapid influx of inflammatory neutrophils. Neutrophil accumulation did not result from contamination of the interleukin-1 with bacterial lipopolysaccharide, nor was it abrogated by treatment with indomethacin, an inhibitor of prostaglandin synthesis. We also observed a small but significant increase in the number of inflammatory macrophages at later timepoints. We went on to show that prophylactic or concomitant administration of interleukin-1 (0.17 ug per mouse) significantly enhanced the resistance of recipient mice to a challenge infection with the facultative intracellular pathogen Listeria monocytogenes. Protection was not caused by contaminating bacterial lipopolysaccharide. Interleukin-1 mediated protection was associated with a rapid burst of serum colony--stimulating activity.

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Subsequent experiments indicated that recombinant human interleukin-1 had a similar ability to enhance anti-listeria resistance. Enhanced resistance occurs in mice of either listeria-resistant or listeria-susceptible phenotype. Administration of recombinant human interleukin-1 or recombinant murine interferon-γ induces a similar enhancement of anti-listeria resistance. Although combined administration of interleukin-1 and interferon-γ had a slight additive effect on anti-listeria resistance at the time of the peak bacterial burden, in general the resistance engendered by both cytokines in combination was similar to that induced by either cytokine alone.

Introduction and Background

A long-term interest of this laboratory has been the investigation of the contributions of inflammation to antibacterial resistance. These studies have utilized a mouse mode of infection with the facultative intracellular pathogen Listeria monocytogenes. Earlier work from this and other laboratories suggested that the ability of the host to mobilize adequate numbers of inflammatory neutrophils and macrophages to foci of infection where these cells could ingest and kill listeriae (8,9) was a major component of both genetically-determined innate resistance (13,14) and immunologically acquired enhanced resistance (8,12,14) to listeria infection. These observations led to our interest in specific inflammatory mediators that might be involved in these host responses. Interleukin-1 is a cytokine, known to be produced by listeria-infected macrophages, with a plethora a biological activities that in general are pro-inflammatory. Although at the time this study was initiated various investigators had suggested a beneficial role for interleukin-1 in host defense, direct evidence for this had not been provided.

The overall objective of this project has been to investigate the contributions of interleukin-1 to inflammation and antibacterial resistance in vivo. Three areas of research were addressed: 1) the ability of interleukin-1 to recruit inflammatory phagocytes in vivo; 2) the ability of interleukin-1 to protect mice against bacterial infection; and 3) interactions between interleukin-1 and other cytokines in the host response to bacterial infection. Initial experiments were performed using purified human interleukin-1 that was purchased from a commercial source. All subsequent experiments were performed with recombinant murine and human interleukin-1α that was generously provided by Dr. Peter Lomedico at Hoffmann-La Roche (Nutley, NJ).

Report of Progress Year 1

Ability of Interleukin-1 to recruit inflammatory phagocytes in vivo

Our initial objective was to examine the ability of interleukin-1 to recruit inflammatory peritoneal neutrophils and macrophages in vivo. This work, which has been published (1), used both purified human and recombinant murine interleukin-1α. Similar results were obtained with both products. In summary, intraperitoneal injection of small amounts of interleukin-1 (1-10 LAF units, 0.2-2.0 ng) resulted in a rapid (peak at 4-20 hours) influx of inflammatory neutrophils (Fig. 1) followed later (60-72 hours) by a small but significant increase in the number of inflammatory macrophages (Fig. 2). Neither cyclooxygenase (indomethacin) nor lipooxygenase (NDGA) inhibitors blocked the IL-1 mediated influx of neutrophils, suggesting that local release of arachidonate metabolies may not be required for this response. When tested in vitro using the under-agarose migration assay, rIL-1 was observed to be at best weakly chemotactic at ratner high concentrations (1000 units per well). This observation, and those of others, leads us to believe that the phagocyte-recruiting activity of IL-1 in vivo may result from the induced release of other mediators by cells in the local environment, rather than from establishment of a directly chemotactic gradient of IL-1.

1

Effects of IL-1 on antibacterial resistance

We next asked whether administration of recombinant murine IL-1α would enhance the resistance of mice to bacterial challenge. This work has been published (2). Injection of mice with graded doses of rIL-1α indicated that approximately 1000 units of rIL-1 (0.2 ug) significantly enhanced the resistance of mice to L. monocytogenes infection (Fig. 3). The best results were obtained when the rIL-1 and L. monocytogenes were injected via the same route (i.v. or i.p.). Maximal protection was achieved when the mice received the rIL-1 concomitant with (i.v. route) or 48 hours before (i.p. route) the L. monocytogenes was injected (Fig. 4). This protective response was not the result of contaminating bacterial endotoxin in the rIL-1α.

Effects of IL-1 on the host response to bacterial infection

In work that has been published (3), we next examined the effects of rIL-1 administration on the course of the host response to L. monocytogenes infection. Our results indicated that there was a 24-48 hour time-lag between administration of rIL-1 and evidence of enhanced resistance by the listeria-infected recipients as measured by clearance of listeriae from the spleen and liver (Fig. 5). Histopathological examination of tissues from rIL-1 treated and control listeria-infected mice indicated that substantially less damage to the spleen, liver and other organs occurred in mice that received rIL-1α. We do not believe that the beneficial effects observed were directly mediated by the rIL-1 that was administered. Rather, we think it more likely that injection of rIL-1 modifies the normal immunoregulatory cascade to accelerate or augment the protective host response. Such a mechanism would likely be reflected in detectable changes in the levels of other cytokines that are released during the host response to infection. Obviously, the possible routes by which rIL-1 might have such an effect are myriad. We have succeeded, through collaboration with Dr. Karen Young, in examining the influence of rIL-1 administration on circulating levels of colony-stimulating activity (CSA), a family of cytokines that may be associated with anti-listeria resistance. We have observed an early (4 hour) significant elevation in CSA in the sera of rIL-1α treated listeria-infected mice (Table 1). It is possible that this early burst of CSA might promote anti-listeria resistance by increasing the production and release of phagocytes from the bone marrow, and perhaps by stimulating the activity of mature phagocytes as well.

Influence of genetically determined anti-listeria resistance on responsiveness to rIL-1

If biological response modifiers such as IL-1 are to become clinically useful, it is important that they work in individuals whose genetic background may make them somewhat less resistant to various pathogens. Innate resistance to L. monocytogenes is documented to be principally regulated by the autosomal incompletely dominant Hc locus on mouse chromosome 2. Because all our previous experiments were performed using the relatively resistant BDF$_1$ mouse strain, we decided to compare the effects of rIL-1 on the anti-listeria resistance of mouse strains of resistant (C57BL/6) and susceptible (A/J, CBA/J, BALB/c) phenotypes. All strains tested demonstrated at least a 1.0 log$_{10}$ increase in resistance in response to rIL-1. This work, which has been submitted for publication (4), suggests that immunotherapy with IL-1 may not be limited by the innate resistance of the recipient, although minor differences in the level of protection evoked by rIL-1 were observed. We also have observed some enhancement of anti-listeria resistance in rIL-1 treated nude mice, thus suggesting that the presence of a mature thymus may not be requisite for some of the beneficial effects of rIL-1 on host defense (5).

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Separate and combined effects of rIL-1 and rIFN-γ on antibacterial resistance

Previous studies have suggested that endogenous release of IFN-γ is required for clearance of listeriae from infected mice, and that administration of high doses of exogenous rIFN-γ elevated anti-listeria resistance. We were interested in determining whether combined administration of rIL-1 and rIFN-γ might have additive or synergistic effects on the expression of anti-listeria resistance. If this occurred it might indicate that rIL-1 and rIFN-γ influence anti-listeria resistance via distinct immunoregulatory pathways. Our results, which have been submitted for publication (6), indicated several points. The first was that human rIL-1α, which was used in these experiments, has an ability to enhance anti-listeria resistance that is comparable in magnitude, and in the dose of rIL-1 required, to what we previously reported for murine rIL-1. We also observed that administration of rIFN-γ (1000-5000 units) substantially enhanced anti-listeria resistance. Although a slightly additive effect of rIL-1 and rIFN-γ was observed under some experimental conditions, in general the protection afforded by rIL-1 and rIFN-γ alone or in combination were virtually identical (Fig. 6). Although these experiments did not indicate any obvious benefits of combined administration of rIL-1 and rIFN-γ, we believe that any eventual clinical applications of cytokines will involve administration of cytokine "cocktails" that contain two or more cytokines with synergistic or complementary modes of action. Experiments are planned that will examine the possible interactions between IL-1 and other likely candidates for increasing host defense such as interleukin-2 and tumor necrosis factor.

Related studies not funded by the Office of Naval Research

Various other projects in this laboratory are addressing experimental problems related to the work described above. We are utilizing the murine listeriosis model for examining the in situ contribution of L3T4+ and LYT2+ T cell populations to anti-listeria resistance. This is done by treating mice in vivo with purified monoclonal antibodies directed against the T cell population of interest before challenging these mice with L. monocytogenes. Our results indicate that anti-L3T4 treatment significantly impairs the resistance of mice to L. monocytogenes as demonstrated by retarded clearance of listeriae from the spleen and liver and increased severity of damage to the liver and other organs (7). Anti-L3T4 treated mice failed to develop delayed type hypersensitivity to listeria antigens in vivo, and their lymphocytes proliferated poorly, and failed to produce IFN-γ, in response to mitogens and antigens, in vitro. Despite this profound decrease in lymphocyte function, anti-L3T4 treated mice succeeded in clearing the initial L. monocytogenes infection and they exhibited a modest increase in resistance to re-challenge L. monocytogenes, although to a much less extent that did control listeria-immunized mice. In the future we hope to determine whether administration of IL-1 or other cytokines might repair the defect in anti-listeria resistance of anti-L3T4 treated mice.

Our laboratory is also involved in studies of IL-1 and other cytokines in a large animal (bovine) system. A student in the laboratory has succeeded in purifying bovine IL-1 from in vitro stimulated monocytes. More importantly, he has shown a substantial species preference (100-1000 fold less LAF units) of bovine thymocytes and fibroblasts for bovine IL-1 over human or murine recombinant IL-1. This observation has been confirmed independently by industrial investigators using recombinant bovine IL-1 (P. Baker, personal communication).

We also are investigating the influence of various cytokines on bovine neutrophil function in vitro. Preliminary results suggest that recombinant human IL-1 and purified natural bovine IL-1 both elicit a weak oxidative burst by bovine neutrophils and prime them for a substantially elevated oxidative response to a second stimulus such as opsonized zymosan.

Relevance of the work performed and future goals

The results of our efforts on our ONR sponsored research has resulted in several publications, in competitive journals, which are among the first reports of the beneficial effects of the administration of IL-1 on host defense. We have also provided evidence for the ability of IL-1 to modulate the immunoregulatory cytokine network that occurs during the host response to bacterial infection. In the coming year we hope to investigate these immunoregulatory interactions in greater detail. These studies will be performed at several levels. 1) As indicated above, we plan on investigating the possible benefits of combining IL-1 with other cytokines; 2) We hope to delineate further the cascade of cytokine activities (by bioassays and immunoassays for the secreted peptides) that result from rIL-1 mediated enhancement of antibacterial resistance; 3) We intend to utilize cDNA probes for IL-1 and other cytokines to demonstrate transcription of genes for various cytokines during bacterial infection.

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Publications resulting from this contract

1. CZUPRYNSKI, C.J., and J.F. Brown. 1987. Purified human and recombinant murine interleukin-1α induced accumulation of inflammatory peritoneal neutrophils and mononuclear phagocytes: possible contributions to antibacterial resistance. Microbial Pathogenesis 3:377-386.

2. CZUPRYNSKI, C.J., and J.F. Brown. 1987. Recombinant murine interleukin-1α enhancement of nonspecific antibacterial resistance. Infect. Immun. 55:2061-2065.

3. CZUPRYNSKI, C.J., and J.F. Brown, A.J. Cooley, and R.S. Kurtz. 1988. Effects of murine recombinant interleukin-1α on the host response to bacterial infection. J. Immunol. 140:962-968.

4. Kurtz, R.S., J.T. Roll, and C.J. CZUPRYNSKI. Recombinant human interleukin-1α enhances anti-listeria resistance in both genetically resistant and susceptible strains of mice. Submitted to Infect. Immun.

5. CZUPRYNSKI, C.J., and J.F. Brown, K.M. Young, A.J. Cooley, and R.S. Kurtz. 1988. Effects of recombinant murine interleukin-1α on the pathogenesis of murine listeriosis. In: M.C. Powanda (Ed.), Monokines and Other Non-lymphocytic Cytokines, Alan R. Liss, Inc., New York, NY, in press.

6. Kurtz, R.S., K.M. Young, and C.J. CZUPRYNSKI. Separate and combined effects of recombinant human interleukin-1α and recombinant murine interferon-γ on antibacterial resistance. Submitted to J. Immunol.

Related publications from the PI's laboratory

7. CZUPRYNSKI, C.J., and J.F. Brown, K.M. Young, A.J. Cooley. Administration of purified monoclonal antibody to L3T4 impairs the resistance of mice to Listeria monocytogenes infection. Submitted to J. Immunol.

8. CZUPRYNSKI, C.J., P.M. Henson, and P.A. Campbell. 1984. Killing of Listeria monocytogenes by inflammatory neutrophils and mononuclear phagocytes from immune and nonimmune mice. J. Leuk. Biol. 35:193-208.

9. Campbell, P.A., C.J. CZUPRYNSKI, and J.L. Cook. 1984. Differential expression o macrophage effector functions: bactericidal versus tumoricidal activities. J. Leuk. Biol. 36:293-306.

10. CZUPRYNSKI, C.J., and J.F. Brown. 1985. Phagocytes from flora-defined and germ free anthymic nude mice do not demonstrate enhanced antibacterial activity. Infect. Immun. 50:425-430.

11. CZUPRYNSKI, C.J., P.M. Henson, and P.A. Campbell. 1985. Enhanced accumulation of inflammatory neutrophils and macrophages by transfer of T cells from mice immunized with Listeria monocytogenes. J. Immunol. 134:3449-3954.

12. CZUPRYNSKI, C.J., B.P. Canono, P.M., Henson, and .A. Campbell. 1985. Genetically determined resistance to listeriosis is associated with increased accumulation of inflammatory neutrophils and macrophages which have enhanced listericidal activity. Immunology 55:511-518.

13. CZUPRYNSKI, C.J., and J.F. Brown. 1986. The relative difference in anti-listeria resistance of C57BL/6 and A/J mice is not eliminated by active immunization or by transfer of listeria-immune T cells. Immunology 58:437-443.

14. CZUPRYNSKI, C.J., and J.F. Brown. 1987. Dual regulation of anti-bacterial resistance and inflammatory neutrophil and macrophage accumulation by L3T4$^+$ and Lyt 2$^+$ listeria-immune T cells. Immunology 60:287-293.

15. Stecha, P., C. Heynen, J.F. Brown, and C.J. CZUPRYNSKI. Effects of the growth of Listeria monocytogenes at reduced temperatures on their resistance to killing by human phagocytes and their virulence for mice. Submitted to Infect. Immun.

16. CZUPRYNSKI, C.J. and J.F. Brown. Augumentation of murine peritoneal macrophage anti-listeria activity by recombinant murine interferon-γ: effects of prior stimulation in vivo, adherence, and time of exposure. Submitted to Infect. Immun.
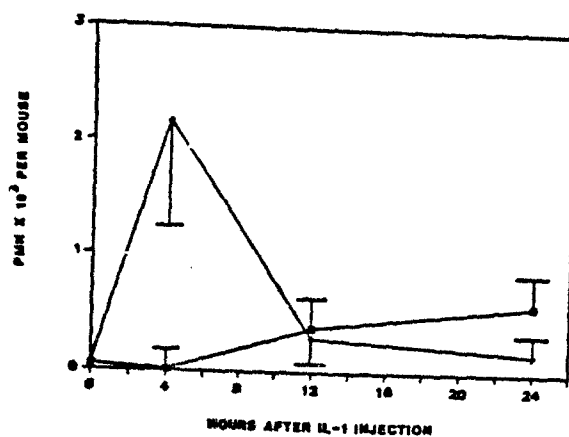
Fig. 1 Time-course of inflammatory peritoneal neutrophil accumulation after i.p. injection of 1 unit rIL-1α (+) or pyrogen-free saline (□). Results are expressed as the mean ± SEM neutrophils ×10⁶ per mouse (4 mice per group).
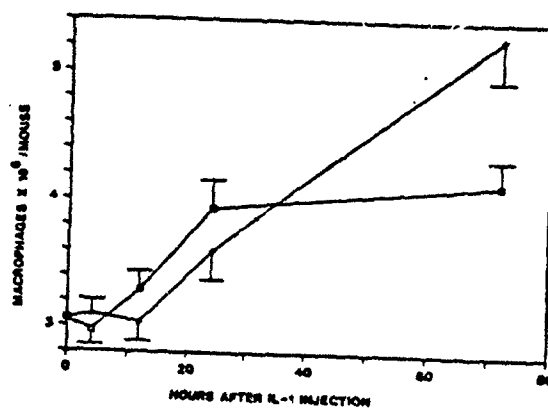

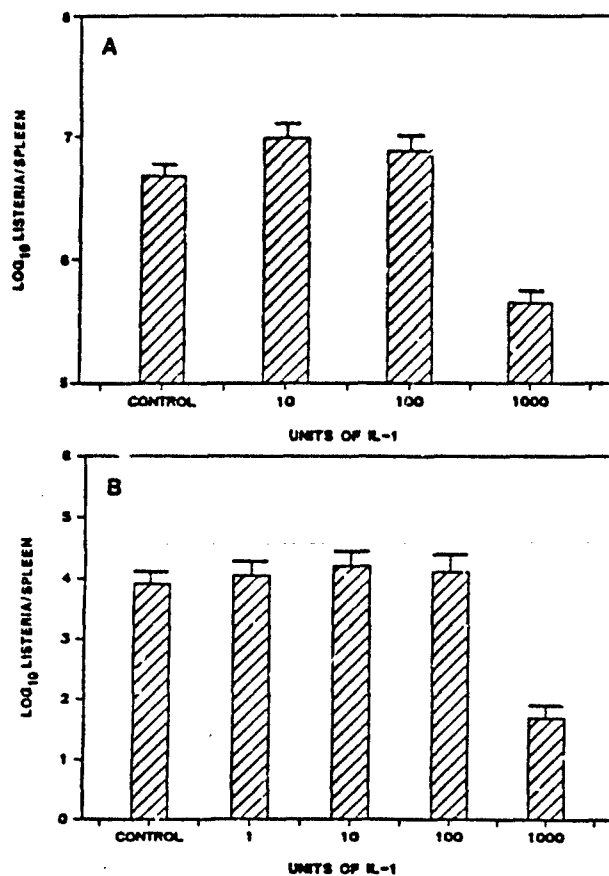**Fig. 2** Time-course of inflammatory peritoneal macrophage accumulation after i.p. injection of 1 unit rIL-1α (+) or pyrogen-free saline (□). Results are expressed as the mean ± SEM macrophages ×10⁶ per mouse (4 mice per group).

FIG. 3 Effect of i.v. (A) or i.p. (B) injection of graded doses (1 to 1.000 U) of rIL-1α on antilisteria resistance. Mice received rIL-1α concomitant with (A) or 4 h before (B) challenge with 2×10^4 L. monocytogenes. Mice were killed 72 h later, and the number of viable L. monocytogenes per spleen was determined. Results are expressed as the mean ± SEM $\log_{10}$ L. monocytogenes per spleen (four mice per group).
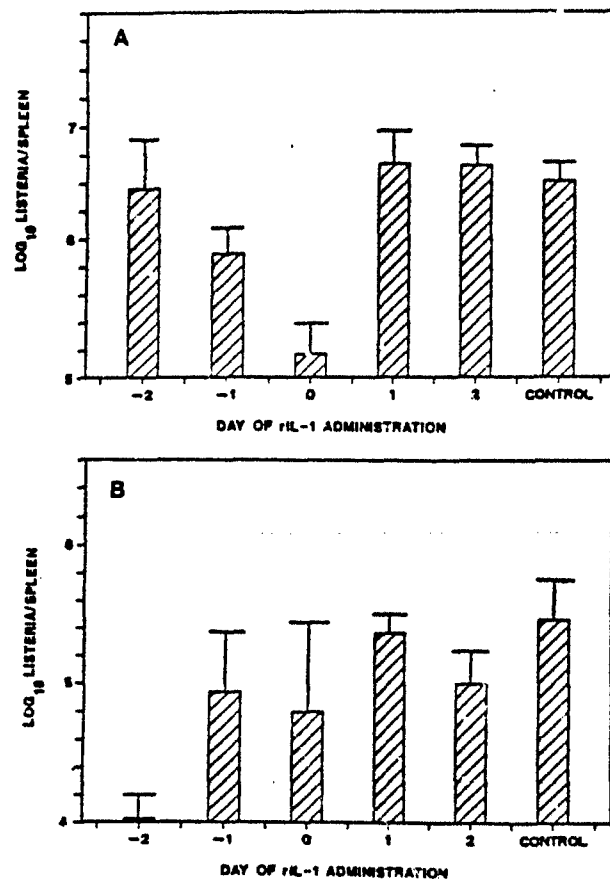
FIG. 4 Effects of timing between rIL-1α administration and L. monocytogenes challenge on antilisteria resistance. Mice received 1.000 U of rIL-1α i.v. or i.p. at the time points indicated (from 2 days before to 2 days after) in relation to the L. monocytogenes challenge. Mice were killed 72 h after challenge, and the number of viable L. monocytogenes per spleen was determined. Results are expressed as the mean ± SEM $\log_{10}$ L. monocytogenes per spleen (four mice per group).

A

B
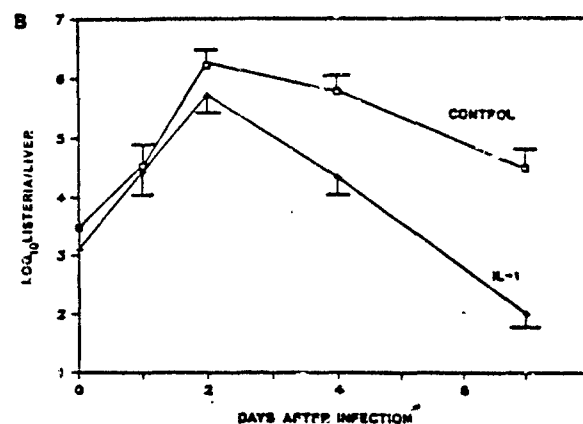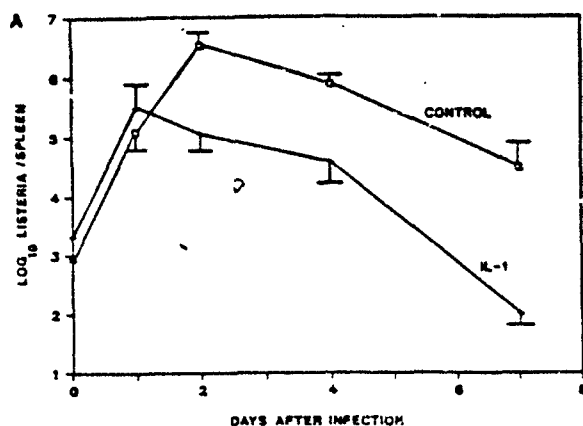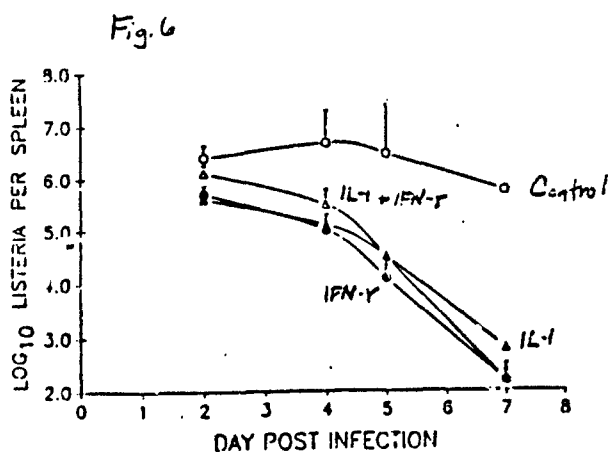
Figure 5. Effects of rIL-1α on the course of a primary sublethal L. monocytogenes infection. Mice were concomitantly injected i.v. with 1000 U rIL-1α and 2 × 10⁴ L. monocytogenes in 0.2 ml pyrogen-free saline; control mice received L. monocytogenes alone. Mice were killed at various times after infection and the $\log_{10}$ viable L. monocytogenes per spleen (A) and liver (B) was determined. The data point at day 0 indicates the number of L. monocytogenes recovered at 4 h after infection. Results expressed are the mean ± SEM of four mice per time point.

TABLE I

CSA in sera from mice at various time points after they were injected i.v. with 1000 U rIL-1α and L. monocytogenes or L. monocytogenes alone

Time after Injection	Mean ± SEM Colonies[a]		Percentages of Colonies[b]	
	rIL-1α + Listeria	Listeria alone	rIL-1α + Listeria	Listeria alone
4 h	65 ± 6	14 ± 1	83	18
1 day	41 ± 2	81 ± 4	53	104
2 days	50 ± 4	74 ± 4	68	100
4 days	25 ± 2	43 ± 3	34	56
7 days	4 ± 1	7 ± 2	6	9

[a] Mean ± SEM of three to five separate cultures. Bone marrow cells from C57BL/6 mice were cultured under soft agar with 0.1 ml of a 1/3 dilution of the indicated sera in a total volume of 1.0 ml for 7 days at 37°C. Granulocyte-macrophage colonies were defined as aggregates of >50 cells.

[b] Mean percentage of colonies obtained as compared with a positive control of WEHI-CM (contains IL-3. see Materials and Methods). Actual colonies obtained for controls were 78 ± 5 for WEHI-CM. 5 ± 1 for normal sera. and none for medium alone.

Fig. 6

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